Vaccine-Induced Antibody Responses as Parameters of the Influence of Endogenous and Environmental Factors

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In laboratory animals, an adequate way to assess effects of environmental exposures on the immune system is to study effects on antigen-specific immune responses, such as after sensitization to T-cell–dependent antigens. This probably also applies to testing effects in the human population. It has thus been suggested that antibody responses to vaccination might be useful in this context. Vaccination responses may be influenced by a variety of factors other than environmental ones. One factor is the vaccine itself; a second is the vaccination procedure used. In addition, the intrinsic capacity of the recipient to respond to a vaccine, which is determined by sex, genetic factors, and age, is important. Psychological stress, nutrition, and (infectious) diseases are also likely to have an impact. We reviewed the literature on vaccine response. With regard to exogenous factors, there is good evidence that smoking, diet, psychological stress, and certain infectious diseases affect vaccination titers, although it is difficult to determine to what extent. Genetic factors render certain individuals nonresponsive to vaccination. In general, in epidemiologic studies of adverse effects of exposure to agents in the environment in which vaccination titers are used, these additional factors need to be taken into consideration. Provided that these factors are corrected for, a study that shows an association of exposure to a given agent with diminished vaccination responses may indicate suboptimal function of the immune system and clinically relevant diminished immune response. It is quite unlikely that environmental exposures that affect responses to vaccination may in fact abrogate protection to the specific pathogen for which vaccination was performed. Only in those cases where individuals have a poor response to the vaccine may exogenous factors perhaps have a clinically significant influence on resistance to the specific pathogen. An exposure-associated inhibition of a vaccination response may, however, signify a decreased host resistance to pathogens against which no vaccination had been performed. *Key words*: age, antibody responses, epidemiology, genetic factors, immunotoxicity, nutritional factors, stress, vaccination. *Environ Health Perspect* 109:757–764 (2001). [Online 31 July 2001]
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Antibody responses to vaccination are influenced by a variety of endogenous factors including genetics, sex, age, and exogenous factors such as stress, nutrition, and infectious diseases. These factors need to be taken into consideration in clinical and epidemiologic studies where the antibody response is the biomarker assessed, for example, when one wants to assess in immunotoxicology investigations effects of exposure to environmental agents.

Studies in laboratory animals have shown that many environmental chemicals exert immunotoxic activity as indicated by altered immune functions, including effects on resistance to experimental infections [reviewed by the International Programme on Chemical Safety (*1*)]. Effects of environmental exposures on immune functions have also been shown in humans (*1*), yet it is less well known whether immunotoxicity induced by environmental chemicals will have such severe consequences for resistance to infections. It has been suggested that where exposure to environmental immunotoxicants may induce subtler immunosuppression, consequences of such suppression may become evident as increased incidences of common infections, such as influenza and common cold (*1*).

In experimental studies in rodents, it has been shown that the antibody response to sheep erythrocytes are a valuable indicator for immunotoxicity (*2*,*3*). This is due to the fact that the humoral immune response to sheep erythrocytes involves major components of the immune system, such as degradation of the erythrocytes by phagocytes, antigen presentation, cellular immune functions resulting in helper activity, and finally production of specific antibodies. In addition, alterations in the response to sheep erythrocytes correlates well with resistance to experimental infectious agents in these animal studies (*3*). This immune function test was also applied in nonhuman primates; for example, exposure of female rhesus monkeys (*Macaca mulatta*) to polychlorinated biphenyls (PCBs) reduced the antibody response to sheep erythrocytes, in conjunction with effects on several other immunologic parameters (*4*).

Of course, it is not possible to use antibody titers to sheep erythrocytes to study immunotoxicity in human populations. It has therefore been suggested that effects of immunotoxic exposures on the specific immune response to agents derived from infectious microorganisms, (e.g., to vaccines) be used instead (*5*,*6*). Depending on the vaccine, major components of the immune system may be involved in the ultimate humoral response to the vaccine. It is not possible for all vaccination responses to translate the vigor of the antibody response to protection. Ideally, vaccination is performed in such a way that those variations in response do not result in altered protection, but protection is not always achieved in every individual vaccinated. Effects of exposure to immunotoxicants on vaccination titers, if and when they are observed, will therefore not necessarily indicate a decreased protection of individuals to the pathogen at which the vaccination is aimed. Rather, this may serve as a model of effects of exposures on immune responses to (other) infectious agents required for proper resistance to (other) infections.

Influence of Environmental Exposures on Resistance to Infectious Diseases and Vaccination Titers

Patients suffering from immune deficiency develop more frequent, more severe, and often atypical infections, depending on the type of the deficiency. Complications of severe immunodeficiency include bacterial, viral, fungal, and parasitic infections. The respiratory tract is a primary target for infectious pathogens, especially in immunosuppressed patients. For instance, infectious complications have been commonly described in patients treated with various cytotoxic drugs for cancer treatment and with immunosuppressants, such as cyclosporin A, for the prevention of allograft rejection or the treatment of autoimmune disorders (*7*,*8*).

Also, such consequences of environmental exposures have been documented in the literature. For instance, children born between July 1978 and June 1987 to mothers that had been exposed to toxic levels of PCBs and dibenzofurans through consumption of contaminated rice bran oil in 1978–1979 showed higher frequencies of upper respiratory tract infections (*5*).

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Exposure to organochlorines through the food chain of Inuit mothers in Nanuvik (Arctic Quebec) has been reported to increase the incidence of otitis media in their breast-fed children (*9*). An association of cumulative background exposure to PCBs and dioxins on the prevalence of otitis media was also reported in a group of 3.5 year olds in the Netherlands (*10*). In addition, an association of the prevalence of chickenpox with maternal exposure was observed.

Few studies have used vaccination titers to detect immunotoxicity in humans. This holds true even for studies of immunosuppression by pharmaceuticals such as cyclosporin. In a study of human antibody production as a response to treatment with murine monoclonal antibodies, decreased anti-mouse antibody production was shown after cyclosporin treatment (*11*). After treatment for acute leukemia, children had reduced antibody responses to diphtheria, tetanus, and inactivated polio vaccine (DT-IPV) (*12*). Also post-transplant (organs, bone marrow) immune suppression has been shown to lead to a long period of hyporesponsiveness to vaccinations (*13–15*).

A vaccination response study that did not involve humans was performed in seals by De Swart et al. (*16*). These authors fed captive seals with herring from the Baltic Sea or from the Atlantic Ocean. The relative contamination of the herring by polyhalogenated hydrocarbons, notably PCBs, was 10-fold higher in Baltic compared to Atlantic herring. In seals that were fed with the Baltic herring, a significantly decreased specific antibody response to ovalbumin was observed.

In a study by Reigert and Graber (*17*), the specific antibody response to tetanus toxoid was studied in 19 children, 12 of which were exposed to lead at concentrations that induced metabolic impairment. The antibody responses appeared unaffected. No other immune parameters were included in that study, so it is unclear whether immunotoxicity occurred in these children, although lead certainly has been identified as an immunotoxicant (*18*).

One example of the role of lifestyle factors in antibody responses in humans is the effects of smoking. Increased specific serum IgA and IgG responses to *Chlamydia pneumoniae* were observed by Von Hertzen et al. (*19,20*). These responses were to the natural infection by the pathogen and not to a vaccine. Hence, alterations in specific antibody titers caused by smoking may be a reflection of effects of the course of the infection and the subsequent antibody titers, rather than a reflection of a direct influence of smoking on the immune response to *Chlamydia*. However, in other studies, smoking has been shown to interact with specific antibody production. Smoking has been implicated in suboptimal responses to vaccination with hepatitis B (*21,22*). In contrast, elevated antibody titers to influenza vaccination were noted in smokers (*23*).

A final example of studies on effects of environmental exposures on specific antibody titers after vaccination is the studies performed in the Netherlands by Weisglas-Kuperus and colleagues (*10,24*). They observed lower antibody responses to measles and rubella in some breast-fed infants. At 3.5 years of age, there was a statistically significant negative correlation of antibody titers to measles vaccination with the exposure to PCBs and dioxins as determined in cord blood, and a statistically significant negative correlation of antibody titers to rubella with maternal exposure to these compounds. However, after correction for sex, early feeding type (formula fed or breast-fed), duration of breast-feeding during infancy, tobacco smoking by one or both of the parents, family history of atopy, and day care or nursery attendance, definitive conclusions could not be drawn.

In a study by Termorshuizen et al. (*25*), an association of season with specific antibody levels after hepatitis B vaccination was established in health-care students. In the course of the vaccination procedure, involving multiple vaccinations, higher antibody titers were observed from the time of the second vaccination onward when the first and second vaccination were applied in the winter as compared to the summer. At the completion of the vaccination regimen, similar levels of antibodies were reached in both study groups. This finding was in accord with the working hypothesis of the authors: exposure to ultraviolet radiation diminished antibody titers after vaccination, and ultraviolet radiation exposure was highest in the summer. Yet, a definitive conclusion on the causal relationship cannot be drawn from this study, as other factors may have had an influence on the vaccination titers.

Variability in Vaccination Titers due to the Vaccination

A number of factors related to the way vaccination is performed determine the qualitative and quantitative immune response to the vaccine. The first is number of vaccine doses (in the case of nonreplicating vaccines). In individuals and in the population, the (average) concentration of antibodies depends on the number of vaccine administrations. More vaccine generally gives higher antibody levels, as reviewed by Halsey and Galaska (*26*), and thereafter confirmed in numerous studies (*27*).

The second factor is spacing of doses. In infant vaccination schedules that have longer intervals between doses, the postvaccination antibody titers are usually higher than in short-spaced vaccination schedules. Short-spaced vaccination series however induce protection earlier (*26–28*). A third factor is vaccine concentration. Most vaccines available are formulated to contain an optimal concentration. Some vaccines result in a better priming and a higher antibody response when a higher dose is given (*27,29*). In practice, this is only relevant for vaccines that are available in a range of concentrations (depending on the indications), such as hepatitis A and hepatitis B vaccines.

Finally, kinetics of the immune response are important. A first dose of an inactivated vaccine often does not induce a detectable antibody response, yet it does prime B- and T-memory cells. There is a vigorous reponse to subsequent booster doses (secondary immune response). Peak levels of antibodies are found 1–3 months after the booster vaccination, then the levels decline. A next dose usually induces a peak response again, and the following decline will end at a higher base level.

Hence, the vaccine, vaccination route, and time point during or after completion of the vaccination procedure will affect the vaccination titers. Therefore, if vaccination titers are used as an indicator in epidemiologic studies, it is important to account for these variables.

Sociogeographic Effects

In some vaccine studies using the same lots of vaccines and schedules, the response in one group is higher than in another group (e.g., in Turkish vs. Belgian infants, in Apeldoorn vs. Rotterdam infants), suggesting that genetic and/or environmental factors affect circulating antibody levels after immunization (*30*).

Similar differences have been found in measles antibody seroprevalences among immunized Inuit, Innu, and Caucasian subjects. Here, too, the higher measles seropositive rate found among native compared to non-native Canadian children may point at genetic as well as environmental factors (*31*), in addition to differences in natural infections that may have occurred in these populations.

Genetically Determined Variability in Vaccination Responses

Two examples of genetically determined variability in vaccination responses have been reported in the literature (*32–45*).

Measles. The relationship of the human leukocyte antigen (HLA) and transporter associated with antigen processing (TAP) genotype with antibody response to measles virus vaccination is shown in Table 1. Generally, nonresponders had higher rates of homozygosity. Regarding HLA class II, nonresponders had a higher homozygosity rate

of the *DR* locus, and excess of *DR7* alleles, a *DRB1* allele, and a *DQA1* allele. Hyperresponders had excess of *DR13* alleles, (another) *DRB1* allele, and (another) *DQA1* allele.

Regarding HLA class I, an association of two *B* alleles with response was found, with an allele dose response in one case, whereas an inverse association was found with two (other) *B* alleles and a *C* allele. Nonresponders were more likely homozygous at a specific amino acid position of TAP2.

Hepatitis B. The relationship of the HLA and complement genotype with antibody response to hepatitis B surface antigen vaccination is shown in Table 2. Regarding HLA class II, nonresponders showed an increased homozygosity for a combination of two alleles. An increased frequency of a wide range of (single) alleles was found, and also combinations of three or four alleles were found. For high responders, increased frequencies of a wide range of (single) alleles was also found, as well as combinations of three alleles. Regarding HLA class I, in nonresponders as well as in high responders, increased frequencies of several (single) alleles were found. Nonresponders showed increased homozygosity for a specific combination of three haplotypes, one for class I, one for class II, and one for complement.

In conclusion, several HLA class I and class II genes are involved in the response to vaccination against measles and hepatitis B. For measles, a polymorphism in TAP may also be involved.

Age and Vaccination Responses

Vaccination response in childhood. Age is an important determinant for the immune response. In infants, maturation of the immune system continues after birth. Neonates are not able to respond to most polysaccharide antigens; children do better after 2 years of age. Also, the response to protein antigens continues to further maturate during the first years of life (*28*). For this reason, infants receive four vaccinations as a basic immunization with DT-IPV, while adults need only three for a similar effect. Circulating antibodies (from maternal origin or from antibodies administered) impair the vaccination response, possibly by neutralizing vaccine antigens or by a suppressor mechanism that downregulates the antibody formation when sufficient antibodies are present. However, circulating antibodies appear not to prevent the antibody responses later in life (*46*). Interpretation of antibody levels as a parameter for the effect of external factors on immune responses needs consideration of this factor, too.

Vaccination responses in the elderly. Using the SENIEUR-protocol (*47*), studies in well-characterized, healthy elderly (> 65 years) populations (including history of illness, infections, drug intake, and laboratory values) have been performed and showed that the serum levels of IgG, IgM, and IgA increase with age, as well as the number of benign monoclonal gammapathies and the number of autoantibodies. The number of lymphocytes and their proliferative activity decreases, while the number of neutrophils increases with aging. Monocytes, basophils, and eosinophils do not change during life, but monocyte function was increased in elderly individuals (*48*,*49*).

As a consequence of age-related alterations in the immune system, the elderly may have an impaired response to primary as well as secondary immunization (*50*). The efficacy of influenza vaccine has been estimated to be 70–90% in young adults, but it is lower in elderly nursing home patients (*51*–*55*). The diminished efficacy has been attributed to lower rates of protective antibody responses against the influenza strains. Hemagglutinin inhibition antibody titers of > 40 are generally considered protective. Yet, several studies indicate that at least 25% of the elderly do not develop hemagglutinin inhibition antibody titers after vaccination (*52*,*56*,*57*).

Following vaccination, elderly, healthy subjects showed reduced production of antitetanus toxoid antibody, compared with young adults. Moreover, the antibody titers of the elderly declined by 6 months to baseline values, whereas in young adults titers persisted for up to 1 year (*58*). A recent study demonstrates that 40% of a population of SENIEUR-compatible Austrians were not protected against tetanus (*59*). Fifty percent of these individuals had been vaccinated within the last 10 years and 25% within the last 5 years.

Especially in the elderly, decreasing effectiveness (*60*) with increasing delay since vaccination has been reported for pneumococcal vaccine (*61*). The overall antibody response among the elderly has been determined to be lower after revaccination than after primary

Table 1. Summary relationship between HLA and TAP and antibody response to measles virus vaccine.

Hyperresponder	Nonresponder	Reference
	Higher *DR* homozygosity rate	(*32*)
	Higher homozygosity rate	(*33*)
	Higher homozygosity rate	(*34*)
Excess *DR13*	Excess *DR7*	(*32*)
Excess *DRB1**13	Excess *DRB1**07	(*35*)
Excess *DQA1**01	Excess *DQA1**05	(*33*)
Association with *B7*, *B51*	Association with *B13*, *B44*, *C5*	(*34*)
B7 allele dose response		(*34*)
	More likely *TAP665* homozygous	(*36*)

Table 2. Relationship between HLA and antibody response to HBsAg (hepatitis B surface antigen) vaccine.

Hyperresponder	Nonresponder/hyporesponder	Reference
Caucasian		
	B8, *SC01*, *DR3* homozygosity	(*37*)
	*DRB1**0701; *DQA1**0201; *DQB1**0201 *DPB**0201	(*38*)
	*DRB1**0701; *DQB1**0202 homozygosity	(*39*)
*DRB1**01	*DRB1**03	(*40*)
*DRB1**15	*DRB1**14	
*DRB1**010*	*DRB1**07	(*41*)
DR5	*DPB1**1101	
*DPB1**040*	*DQB1**020*	
*DQB1**0301		
*DQB1**0501		
*DRB1**13	*DRB1**3 *DRB1**7	(*42*)
*DQB1**0602; *DQA1**0102; *DR15* *DQB1**0603; *DQA1**0103; *DRB1**1301	*DQB1**0604; *DQA1**0102; *DRB1**1302	(*43*)
Japanese		
	Bw54-DR4-DRw53-DQw4	(*44*)
	A, *B*, *DRB1*, *DQA1*, *DQB1*, *DPA1*, *DPB1*	(*45*)
A,B		
B46, B7	*A**2602, *A**1101, *B35*, *B70*	
DRB1		
*08032, *0101, *1403	*0405, *0406, *0802, *0401, *1101	
DQA1	*0302, *0301, *0104, *0601	
DQB1	*0401, * 03032, *0302	
DPA1		
*DPA1**0103		
DPB1		
*0402, *0202, *1301	*1401	

vaccination (*62*). Vaccination procedures in the elderly generally consist of repeat vaccines, and the response to these vaccinations may be less adequate to use in studies of effects of environmental exposures on the immune system.

Effect of Chronic Psychological Stress on the Vaccination Response

Objective scores of chronic stress such as loneliness, psychoneurotic complaints, depression, irascibility, and anxiety have been used to study effects of stress on the immune system in humans (*63–70*). Chronic stress diminishes the efficacy of the immune system to protect the host against infections. Chronic stress leads to a decrease in natural killer cell number and activity, decreased lymphocyte response to mitogens, an increase in CD4/CD8 ratio, and increases in virus infections and antibody titer to latent viruses.

In addition, the impairment of the immune response leads to a poorer response of the immune system to vaccines. The study of the response of individuals to vaccines is preferentially performed by using *de novo* antigens, as these are not affected by previous events. Various groups studied the effect of chronic psychological stress on the antibody response to various vaccines, and variable results were obtained (Table 3). Generally, high levels of stress (negative life events, academic exams, daily stress) and anxiety appear to reduce the antibody response to a primary or secondary immunization with a vaccine.

Jabaaij et al. (*63*) performed a study in stressed subjects characterized by loneliness, daily hassles, psychoneurotic complaints, and submissive coping style. Subjects were vaccinated and antibody titers determined 7 months later. A high stress score derived in the month of the second assessment was associated with a lower antibody response to the vaccine, but in a later, similar study using a higher dose of the same vaccine, no effects were observed at any time point (*64*).

Petry et al. (*65*) vaccinated 81 seronegative subjects with a similar vaccine three times and determined antibody titers 3 months after the third dose (i.e., in the booster phase of immunization). Higher levels of stress, depression, irascibility, and anxiety during the 6-month period following the first vaccination were associated with higher peak antibody titers.

Glaser et al. (*66*) studied the effects of stress on the antibody response to hepatitis B vaccine given three times to healthy students. The "early" seroconvertors, were significantly less anxious and less stressed than "late" seroconvertors, indicating that stress delayed the humoral immune response to hepatitis B vaccination.

Kiecolt-Glaser et al. (*67*) found impaired responses in Alzheimer's caregivers (subject to chronic stress) to influenza vaccination relative to matched controls. One month after vaccination, 65% of the control subjects, but only 37% of the caregivers, had a 4-fold increase in antibody response.

Similar results were recently observed in caregivers of dementia patients receiving a trivalent influenza vaccine (*68*). Mean scores of emotional distress were significantly higher in caregivers than in controls. In 26 of 67 controls (39%), but in only 8 of 50 caregivers (16%), a 4-fold increase in at least one of the IgG subclass titers was observed.

Snyder et al. (*69*) investigated the effect of stress and psychosocial factors on the antibody response to vaccination with KLH (keyhole limpet hemocyanin) antigen. Antibody titers were measured 3 and 8 weeks after immunization and showed that subjects with more stressful events tended to have lower baseline and 3-week postimmunization IgG levels. Psychological distress scores correlated negatively and psychological well-being scores correlated positively with IgG levels. Those who reported less stress tended to have higher IgG levels at 8 weeks postimmunization.

Nutrition and Efficacy of Vaccination

Malnutrition. Protein deficiency can affect immune responses in young children, depending on its severity. Under extreme malnutrition conditions such as marasmic (severe caloric deficiency), and kwashiorkor (severe protein deficiency) impairment of vaccination was found for yellow fever, smallpox (*70*), tuberculosis, and polio (*71,72*). No impairment of the immune response to the vaccination was found under mild and moderate conditions of malnutrition on vaccination against tuberculosis, measles (*73,74*) smallpox (*70,73*), yellow fever (*70*), diphtheria, tetanus, and pertussis (*75*).

Malnutrition caused by anorexia nervosa or bulimia nervosa was associated with disturbances in the immune system (*76,77*). A general decrease in lymphocyte subsets, except for CD19+ cells (B cells) is described for anorexia and bulimia patients. In addition, impairment for the cell-mediated response (delayed-type hypersensitivity) was found in anorexia patients (*76*). It is noteworthy that anorexia nervosa patients are not prone to infections (*78–80*).

Breast-feeding versus formula feeding. Breast and artificial milk are the major nutrition during the first 6 months of life, and still are important in later months. Lesourd (*81*) studied the effect of breast milk and four types of artificial milk on the effect of vaccination. Babies fed breast milk or high-protein cow's milk had an adequate and sustained responses; those fed on formula that was relatively low on proteins and carbohydrates had high but temporary responses, and those fed on low-protein cow's milk or the soy-based formula had poor responses (*81*). Besides protein content, contaminants in the formula may also have had an influence.

Food constituents. The presence or the absence of certain vitamins and nutrients can affect immune responses (*82–85*). Addition of vitamins C and E to food has been shown to stimulate immune responses, and suppressed immune responses have been observed associated with deficiency of vitamins A, B, and E. Immune responses are also affected by iron and zinc deficiencies. These trace metals are essential for the development and maintenance of the cell-mediated (iron and zinc) and humoral response (iron). In general, it appears that cell-mediated and nonspecific immunity are more sensitive to nutrition deficiency than is humoral immunity (*86*).

Currently, there is a growing interest in diets specifically designed to promote health. Probiotics such as lactic acid bacteria can transiently colonize the intestine and exert beneficial effects on the immune system (*87*). Fish oil, which is rich in eicosapentanoic and docosahexaenoic acid, affects cell-mediated and humoral responses in both humans and experimental animals, with some stimulated and others down-regulated (*88,89*).

In the elderly, the effect of immunosenescence is superimposed on the development of malnutrition. Randomized controlled studies have shown that supplementation of vitamin E for 4 months improved certain clinically relevant indices of cell-mediated immunity in healthy elderly persons. Delayed-type hypersensitivity and antibody titers to hepatitis B were significantly increased, as were antibody

Table 3. Effect of stress stimuli in vaccination studies.

Vaccine	Stressor	Observation	No.	Reference
Hepatitis B	Loneliness, hassles	Lower Ab-response	95	(*63*)
Hepatitis B	Daily stress, neuroticism	No effect on Ab-response	68	(*64*)
Hepatitis B	Life events, stress anxiety	Higher peak Ab-response	81	(*65*)
Hepatitis B	Exam stress, social support	Delayed Ab-response	48	(*66*)
Influenza	Alzheimer caregiving	Lower Ab-response	64	(*67*)
Influenza	Depression caregiving	Lower IgG Ab-response	117	(*68*)
KLH	Life events, daily stress	Lower IgG Ab-response	89	(*69*)

KLH, keyhole limpet hemocyanin.

titers to tetanus vaccination (*81,90,91*). In a study of influenza vaccination in the elderly with low serum albumin levels, a very poor antibody response to the influenza vaccination was induced (*81*), but in another study no difference was observed between elderly and young adults (*92*).

In conclusion, the data indicate that nutritional status as well as individual nutrients in food can affect vaccination titers and should therefore be a concern in the design of epidemiologic studies of effects of environmental factors on the immune system.

Influence of Infectious Diseases on the Immune Response to Vaccination

Numerous inflammatory and immune reactions that occur in response to infection might in theory affect the outcome of a vaccination given in the course of that infection. Pathogens may affect the immune response following vaccination by infecting CD4+ Th cells and macrophages. This has been documented for viruses [human immunodeficiency virus (HIV), measles virus, enteroviruses], bacteria (*Streptococci* and *Staphylococci*), and parasites (*Leishmania, Plasmodium*). They may further influence the immune system by stimulating the production of cytokines, which in turn may affect the nature and magnitude of the immune response following vaccination (*93,94*).

Influence of immunosuppressive infections on the vaccination response. HIV, measles virus, some bacteria (*Salmonella*), and helminthes (*Schistosoma, Nematospiroides*) exert well-documented immunosuppressive effects. In addition, it is well known that HIV-infected persons may have a poor response upon vaccination against measles virus and hepatitis A and B virus (*95–98*).

Infection with *Plasmodium* spp. has several effects on the function of immune cells and has been documented to inhibit the antibody response to tetanus toxoid (*99*). Measles virus is clearly immunosuppressive. It interferes with the function of antigen-presenting cells such as monocytes and dendritic cells. This may lead to deficiencies in interleukin-12 (IL-12) production and T cell proliferation (*100–102*). Despite these well-documented immunosuppressive effects of measles virus, the effect of measles virus infection on concurrent vaccination is not documented.

Chronic carriers of hepatitis B virus have a disturbed T-helper cell function, which is associated with a reduced recall response to whole tetanus toxoid (*103*). The effect of chronic hepatitis B virus carriership on primary vaccinations is unknown. The same is true for helminth and bacterial infections. *Salmonella*, *Schistosoma*, and *Nematospiroides* may influence the function of B and T cells (*104–106*). Yet their influence on the vaccination response is not documented.

Influence of nonimmunosuppressive and nonspecified infections on the vaccination response. Oral poliovirus vaccine (OPV), a live attenuated poliovirus, interferes with the antibody response to a rotavirus vaccine. However, the effect was small and could be circumvented by a higher dose of vaccine (*107*).

A number of studies have been directed to the question of whether nonspecific infections, manifested by symptoms such as diarrhea, rhinorrhoea, coughing, fever, rash, or a febrile upper respiratory tract infection, negatively affect the vaccination response against mumps, measles, rubella, and poliovirus. One study described a negative effect (*108*), whereas seven other studies reported no or only minimal clinically significant influence (*109–114*).

Specific interaction with cross-reacting pathogens. Infection with microorganisms that are closely related to vaccine components may interfere with the vaccination response to such components, for example, because they crossreact or limit the replication of vaccine virus. Sabin OPV type 2, for example, interferes with the vaccination response to Sabin type 3 (*115*). Non-polioenteroviruses may also interfere with the vaccination response to OPV. In contrast, nonspecific enteric infection did not interfere with OPV vaccination (*116*).

In conclusion, although some infections may exert well-documented immunosuppressive effects in either humans or laboratory animals, their influence on the vaccination response is poorly documented. The influence of well-known immunosuppressive infections, such as measles virus and HIV, appears limited to well-developed countries such as the Netherlands because their incidence is very low. Clinical measles virus infection in the Netherlands is limited to persons who refuse vaccination on religious grounds. The influence of nonspecific childhood infections on the vaccination response has been evaluated in several studies. These infections appear to have no or only a limited negative influence on the response to vaccination.

Discussion and Conclusion

Vaccination titers are a reflection of the immune function, and changes in vaccination-specific immune responses are therefore considered as indicators for the effect of environmental exposures on the immune system in human populations. To date, few studies have been performed in which vaccination titers were used to detect immunotoxicity in the human population. Hence, there is as yet not much experience with sensitivity of this type of testing in epidemiologic studies. The literature to date indicates that many influences on the response to a vaccination exist, and these therefore should be taken into consideration in the design of epidemiologic studies aimed at assessing the effect of environmental exposures on which the response to vaccination is used as an indicator of the function of the immune system. In other words, these influences need to be carefully controlled for.

It is necessary to know the inter- and intraperson variability in the population regarding the responses to the chosen vaccine, so that the required study group size can be determined. The vaccine itself, the vaccination route, booster vaccination, and time point during or after completion of the vaccination procedure have impacts on the vaccination titers. Therefore, it is important to standardize vaccination procedures. Moreover, insight in the interval between the commencement of the exposure that is being studied and the effect on the immune system that is expected (hours, days, months, years) will help in the study design.

The data indicate that sex, genetics, age, psychological stress, smoking, nutrition, and certain infectious diseases that are not necessarily directly antigenically related to the vaccine all may have an influence on vaccination titers and should be considered as confounders. Also, geographic differences have been noted, which may have several causes, such as socioeconomics or culture. Little information is available on the quantitative relevance of all these confounders, and therefore studies need to be designed so that either these confounders are excluded or so that it is possible to correct for these influences. It is obvious that such influences have differential relevance in different populations, such as elderly versus children or populations in wealthy societies versus underdeveloped regions.

According to the extensive animal studies reported by Luster et al. (*2,3*) the antigen-specific response to T-cell–dependent antigens (sheep erythrocytes) correlates well with host resistance to infectious diseases. It is quite likely that this applies to humans as well. In those cases where reduced antibody titers to a given vaccination are observed that cannot be attributed to any other determinant than the environmental immunosuppressive agent under study, it is likely that the exposed population has lower resistance to infections. Obviously, there is a certain reserve capacity, and not every change in function of the immune system will lead to a decreased resistance in healthy individuals. Yet, since in the entire population a high prevalence of different types of infectious diseases, such as common colds, gastroenteritis, and so on, is evident, further suppression of

immune responses in infected individuals is likely to have an impact. These impacts may be expressed as prolonged duration or more severe disease due to the infection. Such effects may go unnoticed because they may only lead to more of the same symptoms. They may actually not be significant for the individual patient. But due to the high prevalence of many rather innocuous infections, such effects may, on a population basis, be significant.

With respect to the efficacy of vaccination in terms of protection, it needs to be mentioned that it is not always possible to deduce from the vaccination titer the level of protection gained. It is therefore likewise not possible to deduct from effects of environmental factors whether these effects hamper protection against the pathogen at which the vaccination is aimed.

Antibody response after hepatitis B immunization, however, predicts susceptibility to disease on exposure (*117*). This is also true for postimmunization measles antibody responses and for postimmunization polio antibody responses. Responses in the low positive range do not protect against clinical measles when subjects are exposed to the wild measles virus, whereas high levels are protective. A strong correlation exists between low antibody levels after a single dose of (measles) vaccine and high susceptibility to infection with exposure (*118*). So any insult to responses to these vaccines that result in titers below a certain threshold will indicate effects even for the protection at which the vaccine is aimed. It is, however, unlikely that such conditions will usually be encountered. For the majority of the population, vaccination is performed so that modest or even relatively big variations in the response do not result in altered protection, even though not every individual will always be protected. This is corroborated by the findings in developing countries in which malnutrition of children results in impaired responses after vaccination, even though these alterations do not cause the general failure of vaccination strategies (though it should be mentioned that sometimes problems with vaccination to measles are associated with vitamin A deficiency). One may expect that individuals with a response to a vaccine that leads to borderline protection may be subject to experiencing a clinically significant negative consequence of diminished vaccination response if environmental exposure that affects vaccination responses occurs.

In many countries, hepatitis B vaccination is mostly done in adults, generally by three intramuscular injections of the vaccine at 0, 2, and 6 months. Specific antibody titers are generally evident after the second immunization, and maximal titers occur after the third vaccination. Vaccination to measles is mostly done in children. Infants are injected intramuscularly at the age of about 2 months and then at 14 months. For both types of vaccination, nonresponders can be observed, usually < 5%. The choice for these or other types of vaccines obviously depends on the environmental factor that one wants to study, and in what type of study group. This will also determine the magnitude of the effect that is expected. In general, effects of environmental factors on vaccination titers are expected to be modest. Thus, the study group that is evaluated needs to be large enough to have sufficient power to detect such modest differences. Groups of 1–200 individuals have been used. The nature of the environmental agent studied will also determine the design of the study, in particular at what time point antibody titers are measured. Agents that produce reversible effects, such as ultraviolet radiation, may require study of the full kinetics of antibody responses during the entire immunization procedure (e.g., at 2 weeks after each vaccination), whereas persistent chemicals such as PCBs may require titers only after the vaccination procedure has been completed (*10*).

Vaccination titers may prove valuable tools for identifying effects of exposure to immunotoxicants in the human population. However, given the many confounders, even if they are all corrected for, immunotoxicants identified in animals may not induce detectable effects on vaccination titers in humans. Careful consideration of the results of experimental animal studies and epidemiologic studies is then warranted, in terms of exposure, other immune end points, and study size, to evaluate the actual risk that the immunotoxicant poses to humans.

In conclusion, vaccination titers may be applied to study effects of exposures to environmental factors, provided that confounders are adequately controlled for. Variability in the response to vaccination is likely to be smallest in the case of vaccination to an antigen to which no prior exposure, either naturally or by prior vaccination, has occurred, which may apply especially to vaccination in children. In addition, confounders such as stress or smoking may also be less evident in children. For this reason, vaccination in children may prove to be most adequate to study immune effects of environmental factors.

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